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REVIEW ARTICLE

Diagnosing anemia in inflammatory bowel disease: Beyond the established markers

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Abstract

The main types of anemia in inflammatory bowel disease (IBD) are iron deficiency anemia (IDA) and anemia of inflammatory etiology, or anemia of chronic disease (ACD). In the management of IBD patients with anemia it is essential for the physician to diagnose the type of anemia in order to decide in an evidence-based manner for the appropriate treatment. However, the assessment of iron status in IBD in many cases is rather difficult due to coexistent inflammation. For this assessment several indices and markers have been suggested. Ferritin, seems to play a central role in the definition and diagnosis of anemia in IBD and transferrin, transferrin saturation (Tsat), and soluble transferrin receptors are also valuable markers. All these biochemical markers have several limitations because they are not consistently reliable indices, since they are influenced by factors other than changes in iron balance. In this review, in addition to them, we discuss the newer alternative markers for iron status that may be useful when serum ferritin and Tsat are not sufficient. The iron metabolism regulators, hepcidin and prohepcidin, are still under investigation in IBD. Erythrocytes parameters like the red cell distribution width (RDW) and the percentage of hypochromic red cells as well as reticulocyte parameters such as hemoglobin concentration of reticulocytes, red blood cell size factor and reticulocyte distribution width could be useful markers for the evaluation of anemia in IBD.

Contents

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1. Introduction

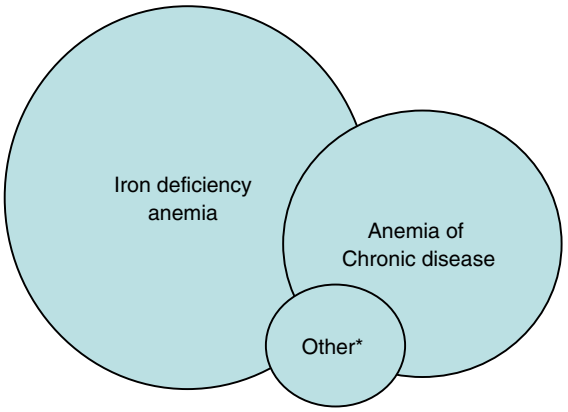
Anemia is the most frequent extraintestinal manifestation of inflammatory bowel disease (IBD) with a great impact on the patients' quality of life.[1] For this reason the research has recently been focused on the pathophysiology, diagnosis and treatment of anemia in IBD. Several studies have contributed in this field unraveling its main mechanisms and suggesting new diagnostic criteria.[2–4] At the same time, new therapeutic methods were developed, especially in the field of iron and erythropoietin supplementation therapy.[5–10] Nowadays, the diagnosis and therapy of anemia has become one of the most challenging fields in the clinical IBD practice.

The prevalence of anemia in IBD varies between 15% and 75%, depending on the definition and subgroup of patients examined.[11] Patients present with a variety of symptoms and signs, such as fatigue, weakness, paleness, headaches, dyspnea, tachycardia, reduced functionality of the musculoskeletal system and impaired cognitive functions.[12,13] These symptoms may occur before the establishment of anemia in the full blood count and the clinicians should detect and treat them early, in order to prevent patient's hospitalization or prolongation of hospital stay. On the other hand, the economic burden of anemia in IBD is very important, on the basis of reducing patient's ability to work and often leading to prolonged hospitalization.

The main types of anemia in IBD are iron deficiency anemia (IDA) and anemia of inflammatory etiology, or anemia of chronic disease (ACD) (Fig. 1). IDA, which is the most common, is the result of reduced iron uptake from the enterocyte and chronic blood loss from the gastrointestinal tract, due to chronic inflammation of the epithelium of the small and large intestine. On the other hand, inflammation, through an inflammatory cytokines-mediated mechanism, leads to a decreased iron level in the circulation and thus to a limited availability of iron for erythroid cells. Besides that, inflammatory mechanisms also lead to decreased iron uptake from the intestinal epithelium, thus providing a very complex two-way interactive pathophysiologic pathway between iron deficiency (ID) mechanisms and inflammation.[4]

In the management of IBD patients with anemia it is essential for the physician to diagnose the type of anemia and to determine the degree of iron deficiency and the degree of inflammation in each patient, in order to decide in an evidence-based manner for the treatment. In our armamentarium nowadays there are plenty of established and new generation indices and markers (Table 1), some of them being a product of latest new technology blood analyzers. There is a growing volume of data about the ability of such markers to differentiate between ID and inflammation, in order to diagnose easily and with a cost-effective way the type of anemia in IBD.

This review discusses the role of established and new indices and markers in the diagnosis of anemia in patients with IBD.



*Vitamin B12 or folate deficiency, drug induced,hemolysis, myelodysplastic syndrome, aplasia, hemoglobinopathies

Figure 1 Etiology of anemia in inflammatory bowel disease.

Table 1 Markers that differentiate iron deficiency anemia from anemia of chronic disease in inflammatory bowel disease.

Parameter	Iron deficiency anemia	Anemia of chronic disease	Mixed anemia
Ferritin	Reduced	Increased or normal	Normal or increased
Transferrin	Increased	Reduced or normal	Reduced
Tsat	Reduced	Reduced	Reduced
sTfR	Increased	Normal or reduced	Normal or increased
sTFR-F index	Increased	Reduced	Increased
Hepcidin	Reduced	Increased	Increased or reduced
MCV	Reduced	Reduced or normal	Reduced or normal
CRP	Normal	Increased	Increased
RDW	Increased	Normal or increased	Normal or increased
CHr	Reduced	Reduced	Reduced
RSF	Reduced	Normal or reduced	Normal or reduced
RDWR	Increased	Normal or increased	Normal or increased

Tsat, Transferrin saturation; sTfR, soluble transferrin receptor; sTFR-F, soluble transferrin receptor-ferritin; MCV, Mean corpuscular volume; CRP, C-reactive protein; RDW, red cell distribution width; RSF, Red blood cell size factor; and RDWR, Reticulocyte distribution width.

2. Iron status parameters

2.1. Serum iron concentration

Iron is an essential element employing by almost all types of cells as a cofactor for fundamental biochemical reactions or as a part of numerous enzymes participating in redox reactions, oxygen transport, energy metabolism and DNA synthesis. Iron possesses a very flexible coordination chemistry and redox reactivity, which enable it to interfere with proteins, bind to oxygen, transfer electrons or mediate catalytic reactions.

The content of iron in the human body is normally 3–4 g, corresponding to a concentration of 40–50 mg of iron/kg body weight. About 60% of the iron is present in the form of hemoglobin in the circulating red blood cells and another 6–7 mg/kg in the form of myoglobin and haem and nonhaem enzymes. Transport iron, bound to the transport protein transferrin, represents only a tiny part (<0.2%) of total body iron. The iron bound to transferrin, supplies the tissues with the iron which they need. The remaining iron (15–30% of the total iron) is bound in a storage form, principally in the cytoplasm in the form of ferritin but also in lysosomes, as hemosiderin.[14]

Serum iron concentrations reflect the balance between the flow of iron into and out of the plasma pool. Iron in this pool turns over very rapidly and plasma iron levels are subject to large and rapid changes. The iron stores show extensive fluctuations dependent on such factors as circadian rhythm, turnover of the iron in the major iron compartments and absorption. The turnover of serum iron takes place many times daily.[15,16] The measurement of serum iron concentration is subject to many variables that may introduce substantial errors into results, such as inadequately processed glassware, contamination of reagents with small amounts of iron, turbidity, and entrapment of iron in plasma proteins during their precipitation.[17] Moreover, many factors such as menstrual bleeding in women,[18,19] acute or chronic inflammatory processes (including IBD),[20,21] malignancy,[22] chemotherapy and myocardial infarction[23,24] may influence the measured values. In conclusion, serum iron concentration is a rather unreliable index for the evaluation of IDA in IBD patients.

2.2. Ferritin

Ferritin is an oligomeric protein characterized by a hollow protein shell capable to store substantial amounts of iron in a non-toxic, soluble and bioavailable form. It serves as a reservoir of iron for cellular requirements for almost all human cells. The main storage sites of ferritin are liver, spleen and skeletal muscle. The origin of serum ferritin is not well established, although animal studies suggest that it is mostly derived from liver.[25]

Measurement of ferritin provides a useful indirect estimate of body iron stores. Small amounts of ferritin secreted into the circulation can be measured by immunoassay. In healthy individuals serum ferritin levels correlate with body iron stores. The maximum possible concentration of serum ferritin (in glycosylated form) derived from active ferritin synthesis is considered to be about 4000 μg/L. Higher concentrations are thought to be due to the release of intracellular ferritin from damaged cells.[26]

In the healthy population elevated ferritin concentrations usually indicate increased iron stores, but a number of disorders may also increase serum ferritin levels independently of the body iron. Serum ferritin is an acute phase reactant and conditions such as fever, acute infections, chronic inflammatory disorders (including IBD), may increase the serum ferritin concentration.[26] Acute and chronic damage to the liver, as well as damage to other tissues, may increase serum ferritin as part of an inflammatory process or by release of tissue ferritin (not glycosylated) from damaged parenchymal cells. Defective clearance of circulating ferritin, for example due to liver dysfunction, may also lead to elevation of the serum ferritin concentration. Malignant diseases and chemotherapy may increase serum ferritin levels.[26] These factors may result in increased serum ferritin levels, which may obscure iron deficiency and complicate the detection of iron overload. On the other hand, conditions that lower the serum ferritin levels without ID are hypothyroidism and vitamin C deficiency.[26]

Every μg/L of serum ferritin corresponds to 8–10 mg storage of iron.[27] Guyatt et al. in an analysis of 55 studies reported that the mean area of the receiver–operator characteristic (ROC) curves for ferritin was 0.95, while that of MCV was only 0.76 in the diagnosis of ID.[28] A ferritin level of ≤12 μg/L has a high specificity (98%) but low sensitivity

(25%) for diagnosis of ID.[29] The sensitivity can be improved to 92% with the same specificity of 98% if a cut-off ferritin level of 30 μg/L is used. Kis et al.[30] in patients who had undergone bone marrow aspirations, found that a ferritin of ≤100 μg/L had a 64.9% sensitivity and a 96.1% specificity for diagnosis of ID. The threshold for ferritin of 100 μg/L was also found to stratify anemic veterans into a high-risk group for advanced colonic neoplasia from a low-risk non-anemic group.[31] Based on these data, a simple clinical cutoff can guide management of anemic subjects: serum ferritin <15 μg/L indicates ID, while serum ferritin >100 μg/L mostly excludes IDA; intermediate values warrant further investigation.[28] Although ferritin level increases with age, and is an acute-phase reactant influenced by chronic inflammation, infection, malignancy and chronic renal failure, the sensitivity and specificity of the serum ferritin is little changed if the 100 μg/L threshold is used.[28,30] Others have proposed that a ferritin level of 70 μg/L was the necessary safety limit for exclusion of ID.[32,33] Guagnozzi et al.[34] in a study with 76 IBD patients found that the sensitivity and specificity of ferritin, with a cutoff value of 15 ng/mL, were low (89% and 64.5%, respectively) for diagnosis of ID. ROC analysis demonstrated that ferritin diagnostic accuracy could be improved by using higher cutoff values. In anemic IBD patients, the ferritin cutoff value of 28 ng/mL showed a sensitivity of 93.8% and a specificity of 90%, indicating that ferritin may be proposed as an accurate, simple, and useful marker to identify ID, particularly in anemic IBD patients.

According to the established guidelines on the diagnosis and management of IDA and anemia in IBD,[35] serum ferritin is included in the screening parameters for anemia, with the recommendation to be measured every 6 to 12 months, in patients in remission or mild disease, or at least every 3 months in outpatients with active disease. Ferritin was added to other parameters (full blood count and CRP) as minimum requirements to diagnose anemia, inflammatory flare or IDA in an early stage, because IDA is a very common nutritional deficiency with a strong impact on anemia.[36] Ferritin, along with Tsat and CRP, is the minimum workup for anemic patients with IBD. The combination of serum ferritin levels with soluble transferrin receptors (sTfR) levels can be used to detect ID (increased sTfR, low ferritin), inflammation (normal sTfR and ferritin) or mixed conditions (increased sTfR, normal ferritin). Furthermore, ferritin <30 μg/L (in combination with Tsat <16%) is an established index for ID in patients without evidence of inflammation. In the presence of inflammation, the lower limit of serum ferritin consistent with normal iron stores is considered to be 100 μg/L, thus the diagnostic criteria for ACD are a serum ferritin >100 μg/L and Tsat <16%. If the serum ferritin level is between 30 and 100 μg/L, a combination of true IDA and ACD is likely. Finally, serum ferritin can be used in the form of the sTfR/log ferritin ratio as a useful tool to exclude ID, when it is <1.[37]

2.3. Transferrin/total iron binding capacity

Transferrin (TRF) mediates iron exchange between body tissues, taking iron from donor sites, such as the gut and macrophages, to acceptor cells like erythroblasts. In conditions of normal iron status, serum TRF is saturated to about one-third of its iron-binding capacity, so that we have a mixture of apotransferrin, the two mono-ferric forms and di-ferric TRF. TRF is mainly synthesized in liver hepatocytes and in small amounts in brain, lymph nodes, testicular tissue and mammary glands.[38]

Serum TRF is an indicator for ID but is not as useful as the serum ferritin level. TRF concentrations increase when iron stores are depleted and decrease with iron overload. However, the TRF level is not a consistently reliable index, since it is influenced by factors other than changes in iron balance. Inflammation, infection, malignancy, liver disease, nephrotic syndrome and malnutrition may all reduce the serum TRF concentration, while pregnancy and oral contraceptives will increase it.[26]

Total iron binding capacity (TIBC) indicates the maximum amount of iron needed to saturate plasma or serum TRF. The correlation between TIBC and TRF is generally considered good, but they also present important differences. Although TIBC is cheaper, there are several advantages of serum TRF. Due to binding of iron to other plasma proteins (mainly albumin), TIBC methods generally overestimate the iron binding capacity of transferring and no generic reference values are available. In contrast, internationally accepted interim reference ranges are available for serum TRF. In view of these observations, determination of TRF concentration, rather than TIBC, is recommended. However, TIBC measurements may be preferred in populations with genetic variation of TRF.[39]

Although serum ferritin measurement is the investigation of choice in ID, many laboratories continue to measure iron and TIBC/TRF. In a recent study, Hawkins et al. comparing the diagnostic utility of iron, TRF and Tsat measurements in the diagnosis of ID across inpatient and outpatient found that TRF or TIBC measurement outperformed iron measurement and saturation index.[40] Shek et al.,[41] in a more cost effective approach to the diagnosis of ID, suggested that serum iron and TIBC should be done first, and serum ferritin is not required when Tsat <16% and TIBC >70 mmol/L or if Tsat is ≥22% and TIBC ≤70 mmol/L. A study from Germany[42] investigating the diagnostic validity of an increased TRF concentration for diagnosis of ID showed diagnostic sensitivity 36% and specificity 97%. Finally, a recent study on rheumatoid arthritis showed limited value of TIBC in the diagnosis of ID.[43]

In conclusion, it seems that TRF/TIBC test is superior to serum iron or Tsat, but is not better than ferritin in diagnosis of ID. In any way, TRF/TIBC should be used with consciousness and only in parallel with other iron status tests, in order to get the highest diagnostic yield. In IBD patients, TRF has been suggested to be included, among other parameters, in the more extensive workup of anemia, when the minimum investigation (ferritin, Tsat, and CRP) cannot identify the cause of anemia.[35] In addition, TRF levels can be used, in combination with other indices (serum erythropoietin and sTfR), to predict cases that will not respond to intravenous iron alone and may profit from a combination therapy with erythropoiesis stimulating agents.[44]

2.4. Transferrin saturation

Transferrin saturation (Tsat) is an index reported as a percentage and is the quotient of iron levels (μmol/L)/TRF

levels (mg/dl) in the serum or plasma multiplied by 70.9 (fasting blood sample).[45] Tsat is a measurement of the iron content of the circulating TRF. Normally, there is enough TRF present in 100 mL serum to bind 250 to 450 μg (4.4 to 8.0 μmol) of iron. Since the normal iron levels are about 1.8 μmol/dl (100 μg/dl), TRF is roughly one-third saturated with iron. TRF is normally 20 to 50% saturated with iron. A normal value for Tsat often accompanies low serum iron levels in the ACD. However, exceptions are so common as to considerably detract from the diagnostic value of measuring Tsat.[17]

A Tsat <16% implies a suboptimal supply of iron for erythropoiesis. A reduced Tsat has a relatively high sensitivity (90%) but a relatively low specificity (40–50%) for detecting ID.[46] Determination of Tsat gives only an indirect indication of the extent of iron use in the bone marrow and does not provide any information about the condition of the iron stores. As Tsat is subject to certain circadian fluctuations, its measurement should always be carried out at the same time of the day and repeated several times.[45] Tsat also has some acute-phase reactivity as TRF may be elevated in the setting of inflammation, which would lower the Tsat, if circulating iron is constant. Decreased TRF synthesis in the setting of malnutrition and chronic disease results in a raised Tsat.[46]

Three studies[47–49] examining the use of Tsat in renal failure patients, with cutoff values between 19 and 21%, showed sensitivity 59–88% and specificity 63–78% in diagnosis of ID, suggesting that only a few patients with true ID have a Tsat >20%.[46] In the guidelines for diagnosis of anemia in IBD, Tsat is included in the minimum anemia workup, along with ferritin and CRP. It is used, in combination with ferritin, at a lower cutoff point (Tsat <16%) in the diagnostic criteria for IDA (when ferritin is <30 μg/L) or for ACD (when ferritin is >100 μg/L). A Tsat level between 16 and 50% indicates adequate iron stores, while a value >50% is consistent with a potential iron overload.[35]

2.5. Soluble transferrin receptors

Serum iron, in the form of diferric TRF, is delivered to cells via the TRF-to-cell cycle, which involves the transferrin receptor (TfR1), a disulfide-linked homodimer composed of two identical glycosylated subunits. Each of the subunits can bind one diferric TRF molecule. The number of soluble transferrin receptors (sTfR) reflects the cellular requirements for iron, and varies both as a function of the cell type, and also with the cell's morphological development. Whereas early normoblasts have some 300,000 TRF receptors, at the peak period of haem synthesis, in the intermediate normoblast, this increases to around 800,000/cell. The circulating receptor seems to be a truncated form of the cellular receptor and it is bound to TRF.[25]

In ID the numbers of TfR increase significantly. Part of the TfR is shed into the plasma, where the concentration can be measured by immunoassay and used as an indicator of ID. This assay seems particularly useful for diagnosis of ID in patients with infection, inflammation or malignancy where serum ferritin is not a good indicator of IDA.[50] The concentration of sTfRs in the serum is an indicator of the iron supply available for erythropoiesis; sTfRs reflect erythropoiesis and inversely the amount of iron available for erythropoiesis.[45,51–53]

Unlike ferritin and TRF, chronic inflammation and hepatic damage have no effect on sTfRs, which should make them a more reliable parameter than serum ferritin for diagnosing ID in patients with IBD.[45,46] However, Fernandez-Rodriguez et al.[54] found sTfRs to be less accurate than serum ferritin in this setting. They demonstrated a sTfR sensitivity of 70% and a specificity of 59% at a cutoff of 2.6 mg/L. Tessitore et al.[48] demonstrated a sTfR sensitivity of 81% and a specificity of 71% at a cutoff of 1.5 mg/L. The sTfR:log ferritin ratio has also been suggested as able to differentiate more accurately between IDA and ACD or to assess the iron status in patients with mixed type anemia.[53] In another study of 176 patients (51.1% with ACD and 48.8% with IDA),[55] both the sensitivity and specificity of sTfR in IDA was found to be 100%, whereas in ACD, these were 66.6% and 100% respectively, concluding that sTfR is a reliable index of IDA and could be useful in distinguishing IDA from ACD. These findings are in agreement with a more recent study[56] where it was found that sTfR levels can be very useful in differentiating pure IDA, ACD and ACD with coexisting IDA, thus providing a noninvasive alternative to bone marrow iron. A high diagnostic power of sTfR or sTfR:log ferritin ratio for differentiating IDA from ACD and mixed anemia has been suggested by several studies in various groups of patients.[37,57]

Increased concentrations of sTfRs are reported in other disorders of erythropoiesis, such as hemolytic anemia, thalassemia and polycythemia, while they are reduced in aplastic anemia and other conditions with hypoproliferative erythropoiesis, such as renal anemia.[45] Serum sTfR levels average 5.0 ± 1.0 mg/L in normal subjects but the various commercial assays give disparate values because of the lack of an international standard.[52] The most important determinant of sTfR levels appears to be marrow erythropoietic activity which can cause variations up to 8 times below and up to 20 times above average normal values. Measurements of sTfR are very helpful to investigate the pathophysiology of anemia, quantitatively evaluating the absolute rate of erythropoiesis and the adequacy of marrow proliferative capacity for any given degree of anemia, and to monitor the erythropoietic response to various forms of therapy, in particular allowing to predict response early when changes in hemoglobin are not yet apparent.[52] Conclusively, sTfR represents a valuable quantitative assay of marrow erythropoietic activity as well as a marker of tissue ID, with the restriction that the assay is not widely available and not standardized, which impedes its clinical application.

3. Iron metabolism regulators

3.1. Hepcidin

Hepcidin is a circulating peptide which plays a major role in iron homeostasis. It is mainly produced in the liver as well as by myeloid cells[58] and by activated splenocytes,[59] through a precursor protein (prohepcidin). Hepcidin reduces the quantity of circulating iron by preventing its exit from the cells, especially from enterocytes and macrophages. It binds to ferroportin, in order to limit iron egress, inducing its internalization and degradation.[60,61] In the absence of

hepcidin, increased intestinal iron absorption associated with increased iron efflux from macrophage leads to parenchymal iron overload.[62,63]

Hepcidin expression is controlled by iron and inflammation.[64] The proinflammatory cytokines play a central role in the induction of the hepcidin gene.[65] IL6 stimulates hepcidin expression in vivo with concomitant reduction in serum iron.[66,67] The characteristic features of ACD (reduction in serum iron, retention of iron in macrophages and blocking of intestinal iron absorption) are all compatible with the consequences of an increase in the production of hepcidin. Iron overload induces an increase in the synthesis of hepcidin whereas IDA results in the reduction in the production of hepcidin, thus ensuring a better availability of iron to the developing erythrocytes in the bone marrow.[68] Hypoxia also inhibits the synthesis of hepcidin.[65] It seems that hepcidin appears as the "ferrostat" of the organism, adjusting the quantities of circulating iron according to body requirements.

The literature about the role of hepcidin in the mechanisms of anemia in IBD is limited[69–71] and not very clear with the results of the existing studies to be rather conflicting (Table 2). In the study of our group[71] the sensitivity of low hepcidin for diagnosis of anemia was 71% and the specificity was 43%. Similarly, the sensitivity of low hepcidin for diagnosis of IDA was 81% and the specificity was 45%.

It is worth mentioning that currently there is no reference method for hepcidin measurement. Hepcidin levels reported by the various methods vary considerably but analytical variance is generally low and similar for all methods.[72]

3.2. Prohepcidin

Prohepcidin is the 60 amino acid immature form of hepcidin. Serum prohepcidin levels have controversial clinical significance since they have been found to be highly variable in normal physiological conditions[73] and without correlation with the expected hepcidin responses to physiologically relevant stimuli.[74] Sasu et al.[75] reported that prohepcidin did not correlate with hepcidin or anemia of inflammation and appears to be an unstable analyte in serum.

Research work on the role of prohepcidin in the anemia of IBD patients is very limited[71,76,77] (Table 2). In the study of our group,[71] median prohepcidin levels were significantly lower in IBD patients compared with HC ($P=0.03$), but after adjustment with other covariates no correlations with parameters of iron status or disease activity were found.

It seems that both hepcidin and prohepcidin offer a major contribution in the development of anemia in IBD, but their levels alone seem inadequate for use in distinguishing IDA from ACD.

Table 2 Studies evaluating hepcidin and prohepcidin in inflammatory bowel disease.

Author (reference)	Study design	Disease (N) HC (N)	Results
Semrin et al. [69]	Urine hepcidin measurement	CD (19)	Increased levels in active disease positively correlated with IL-6 and CRP
Arnold et al. [70]	Serum hepcidin measurement	UC (51) CD (10) HC (25)	Decreased levels positively correlated with IL-6
Oustamanolakis et al. [71]	Serum hepcidin measurement	UC (49) CD (51) HC (102)	Increased levels positively correlated with ferritin and disease activity (for UC)
Kaya et al. [76]	Serum prohepcidin measurement	Pediatric IBD (15)	Increased levels positively correlated CRP
Nagy et al [77]	Serum prohepcidin measurement	UC (72) CD (30) HC (28)	No significant different compared with controls
Oustamanolakis et al [71]	Serum prohepcidin measurement	UC (49) CD (51) HC (102)	Decreased levels not correlated with ferritin or disease activity

CD, Crohn's disease; UC, ulcerative colitis; HC, healthy controls; CRP, C reactive protein; and IL-6, interleukin-6.

4. Erythrocytes parameters

4.1. Red cell distribution width

The red cell distribution width (RDW) is derived from pulse height analysis and is the width of the red cell size distribution curve in fL at the 20% level of the peak. The RDW can also be expressed as the CV% of the measurements of the red cell volume. It is a quantitative measurement of variation in red cell size and is equivalent to anisocytosis seen on the examination of a stained blood film.[78] A classification of anemia based on MCV and RDW has been suggested.[79] In addition to microcytic, normocytic, and macrocytic, this classification further divides the RBC population into homogeneous (with normal RDW) and heterogeneous (with increased RDW). The former include hypoproliferative anemia, aplasia, and thalassemia heterozygosis; the latter comprise nutritional anemia such as deficiencies in iron, B12, and folic acid and sideroblastic anemia.[80] However, an increase in the RDW in patients with anemia due to chronic infections and at least half of heterozygotes for thalassemia have been reported, and normal values of RDW are seen in 15%–20% of IDA.[80] Where microcytosis and macrocytosis exist within the same sample, the two abnormalities may cancel each other out and cause a normal MCV, however, the resulting high RDW will identify the error.[78] Moreover, in patients treated with purine analogs the effect of these drugs on MCV should be taken

into account. Both MCV and RDW should be validated in this type of patients.

There is a wide distribution of RDW values within a given disease and this has diminished its usefulness in differential diagnosis, but its importance as a general marker of abnormality has been maintained.[81] Moreover there are significant differences on the measurements (CV percentage or direct measurement) and the reference intervals in the various methods used for calculation of the RDW.[80]

The amount of literature concerning the implication of RDW in the field of IBD is rather limited. Cakal et al. in 2009[82] found that RDW was significantly elevated in patients with active IBD compared with those with inactive disease and controls ($P<0.05$). In another retrospective review of 284 patients with IBD,[83] there was a significant difference in the mean RDW between CD and UC (14.9 vs. 14.3, $P=0.027$), suggesting RDW as a marker in differentiating CD from UC. In a study from our group[84] RDW was significantly increased in IBD patients compared with HC, whereas it was positively correlated with sTfR and negatively with Tsat. Additionally, RDW was found significantly different in patients with IDA compared with those without IDA. High RDW (>14) was among the best markers for diagnosis of IDA with a sensitivity of 93% and a specificity of 81%. Finally, RDW was not significantly correlated with disease activity.

4.2. Percentage of hypochromic red cells

Percentage of hypochromic red cells is defined as cells with intracellular hemoglobin of <28 g/dl.[85] In the healthy population, the percentage of hypochromic red cells does not exceed 2.5% and values greater than this are indicative of iron deficient erythropoiesis.[86] Percentage of hypochromic red cells is the concentration of hemoglobin in individual cells rather than the mean, as happens with mean cell hemoglobin (MCH) or mean cell hemoglobin concentration (MCHC). It is a more sensitive marker because small changes in the number of red cells with inadequate hemoglobin can be measured before there is any change in the MCHC.[78] Some sophisticated instruments can report this parameter but it has been argued that, as mature red cells have a longer lifespan, the percentage of hypochromic red cells integrates information from over too long a period and may be less sensitive than reticulocyte hemoglobin in diagnosing functional IDA or monitoring anemia treatment.[78] Some studies report that the percentage of hypochromic red cells is sensitive enough for the measurement of functional IDA.[87] Additionally, the utility of the test is limited as the percentage of hypochromic red blood cells is dependent on the total number of red blood cells, which may vary with the length of storage time. Currently, there are no studies investigating the role of this parameter in IBD.

4.3. Erythrocyte zinc protoporphyrin

In IDA and lead poisoning, the enzyme ferrochelatase catalyzes the incorporation of zinc, instead of iron, into protoporphyrin IX (the immediate precursor of heme), resulting in the formation of zinc protoporphyrin (ZPP). As the levels of ZPP reflect iron status in the bone marrow during erythropoiesis, ZPP values >40 μmol/mol hemoglobin have been shown to indicate in addition to IDA, its severity as well and thus distinguish between mild (latent) IDA without clinical symptoms (ZPP: 40–60 μmol/mol hemoglobin) and IDA with clinical symptoms (ZPP >80 μmol/mol hemoglobin).[45] ZPP is also valuable in the diagnosis of ACD.[88] However, as zinc deficiency rather common in inflammatory disorders,[89] the interpretation of ZPP levels should be handled with caution in the setting of IBD.

5. Reticulocytes parameters

5.1. Mean reticulocyte volume

There is evidence that Mean Reticulocyte Volume (MRV) increases after iron supplementation therapy in patients with IDA and decreases with the development of iron deficient erythropoiesis. The MRV decreases and reticulocytes are smaller than mature red cells following treatment with vitamin B12 or folate.[78] However there are limitations in the use of MRV since it lacks standardization, which means numeric results from different manufacturers are not comparable and there is no quality control material available.

5.2. Reticulocyte hemoglobin concentration

After the decade of 1990 some new technology hematology analyzers made possible to measure the hemoglobin concentration of reticulocytes (CHr). CHr is measured in the stained reticulocytes using two angle light scatter. The reference mean value in healthy population is 30.8 pg, with the same value in males and females, and the lower limit of normal is 28 pg.[78] The CHr provides an indirect measure of the functional iron available for new red blood cell production over the previous 3–4 days and it also provides an early measure of the response to iron therapy, increasing within 2–4 days of the initiation of intravenous iron therapy.[78] It is an early indicator of iron-restricted erythropoiesis in patients receiving erythropoietin therapy. These patients may have functional IDA and respond to iron therapy even with very high serum ferritin values. A value of CHr <28 pg was found to predict functional IDA more accurately than ferritin or Tsat.[78]

Although there are no studies evaluating CHr in patients with IBD it could be a reliable index for measuring the response to iron supplementation therapy. Large studies are needed to confirm this hypothesis in this particular group of patients.

5.3. Immature reticulocyte fraction

Immature Reticulocyte Fraction (IRF) indicates the less mature subgroup of reticulocytes, which contain the most RNA, and is an early and sensitive index of erythropoiesis.[90] Immature reticulocytes are released into the peripheral circulation during periods of intense erythropoietic stimulation such as hemorrhage, certain anemias or in response to therapy to stimulate bone marrow production.[78] The IRF increases before the total reticulocyte count and has been found to be useful in distinguishing anemias characterized by

increased marrow erythropoiesis (high reticulocytes, high IRF) from anemias due to reduced marrow activity (low reticulocytes, low IRF) and from situations such as acute infections and myelodysplastic syndromes (low/normal reticulocytes, high IRF).[80]

A recent study, evaluating IRF in IBD patients,[84] showed significantly increased IRF in IBD patients compared to HC ($p<0.0001$). IRF was not significantly correlated with ferritin, Tsat or sTfR and no differences in IRF between patients with IDA and patients without IDA were found. Higher IRF in patients compared to HC could be attributed to an ongoing blood loss in IBD patients, often subclinical, as a result of chronic inflammation in the gut, thus leading to an increased "drive" for erythropoiesis in the bone marrow, even in low iron availability conditions, due to impaired iron absorption.

Limitations of IRF are the non-standardization, the reference intervals are method dependent and the sensitivity is lower in non-fluorescence-based analyzers.[80] This makes IRF a marker of rather limited use in anemia evaluation in IBD.

5.4. Red blood cell size factor

Red blood cell size factor (RSF) is the result of the square root of the product of Mean corpuscular volume (MCV) multiplied by the Mean Reticulocyte Volume (MRV) and seems to be a suitable parameter for the study of bone marrow erythropoietic activity, as it provides a very good level of agreement with reticulocyte hemoglobin content (CHr). There is evidence of a significant correlation between RSF and CHr with excellent diagnostic performance of RSF compared with CHr in diagnosing of the type of anemia.[91] A significant correlation between RSF and reticulocyte hemoglobin equivalent (Ret He) in the diagnosis of inefficient erythropoiesis has also been observed.[92]

RSF was found significantly positively correlated with Tsat and negatively with sTfR in a recent study of 100 patients with IBD.[84] RSF was significantly lower in patients with IDA compared with patients with other causes of anemia and patients without anemia. Low RSF was among the best markers for diagnosis of ID with a sensitivity of 83% and a specificity of 82%. On the contrary, concerning disease activity, RSF was not significantly different between active and non-active disease and no significant correlation between RSF and CRP levels was found. Based on these data RSF seems to be a sensitive real time parameter for the early detection of the impaired erythropoietic mechanisms in IBD patients.

5.5. Reticulocyte distribution width

Reticulocyte Distribution Width (RDWR) is a new generation reticulocyte index, which is automatically reported in the new technology hematology analyzers. It can be presented as RDWR-CV (Coefficient of Variation) and RDWR-SD (Standard Deviation). RDWR-SD is the standard deviation of the retic volume multiplied by the volume factor and is expressed in femptoliters (fL). RDWR-CV is the ratio of RDWR-SD and the retic volume mean (MRV) multiplied by 100 and is expressed as percentage. Both RDWR-SD and RDWR-CV are derived from the reticulocyte histogram. RDWR is an indication of the size dispersion within the reticulocyte population.[93]

In a recent study both indices were found to be significantly increased in patients with CD and UC, compared to HC ($p<0.0001$).[84] RDWR-CV only was significantly negatively correlated with Tsat and positively with sTfR. In addition, RDWR-CV was significantly higher in patients with IDA compared with patients with other causes of anemia and patients without anemia. The sensitivity of high values of RDWR-CV for diagnosis of IDA was 60% and the specificity was 51%. It seems that RDWR and reticulocyte number play a similar role for reticulocytes as RBC count and RDW play for red blood cells. Both RDWR-SD and RDWR-CV were significantly correlated with disease activity and CRP.

6. Conclusions

A great challenge for the IBD practitioner is to combine traditional/conventional anemia markers with the new generation parameters, in order to achieve the highest diagnostic yield of anemia, in patients with IBD (Fig. 2). Anemia is a rather complex manifestation of IBD, due to the mixed type of IDA and ACD.

From the conventional markers, serum iron remains an unreliable index for the diagnosis of IDA, because it is affected by a variety of factors. Ferritin, seems to play a central role in the definition and diagnosis of anemia in IBD and, in combination with other parameters, remains a cornerstone of the diagnosis of IDA, with or without ACD. TRF is superior to serum iron or Tsat but not better than ferritin in diagnosis of IDA; however, it can be used with other markers to detect patients who can profit from combination therapy with iron and erythropoietin. Tsat is a classic marker of anemia, giving indirect information of the extent of iron use in the bone marrow, although it does not provide information about the condition of iron stores. It is included in the definition of anemia in IBD, as well as in the minimum workup, in anemic patients with IBD. sTfR indicate marrow erythropoietic activity and reflect tissue IDA; remains useful in the differential diagnosis between IDA and ACD, but is expensive and not always available.

Iron metabolism regulators, hepcidin and prohepcidin, are still under investigation for their role in the anemia of IBD, they seem to play a central role in the development of it, but they are rather inadequate to distinguish IDA from ACD.

In the group of erythrocyte parameters, RDW is a classic and very strong marker for diagnosis of IDA, with good values of sensitivity and specificity, but not very useful in differential diagnosis, remaining a rather general marker of abnormality. Percentage of hypochromic red cells and zinc protoporphyrin are not validated in patients with IBD, with the former still being unclear from the supporting literature whether it is better or worse than CHr in diagnosing functional IDA. A recently described mathematic model[94] suggesting new ways of diagnosing and predicting IDA, with the use of erythrocyte parameters, could be evaluated in IBD.

As far as the new generation reticulocytes parameters are concerned (MRV, CHr, IRF, RSF and RDWR), the literature about their role in the anemia of IBD is very limited, but some

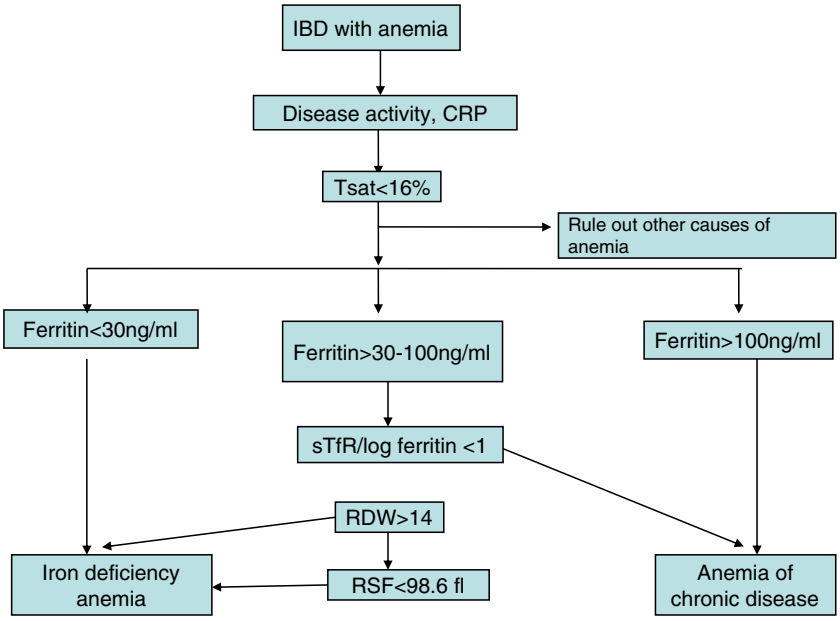


Figure 2 Algorithm for the differentiation of iron deficiency anemia from anemia of chronic disease in inflammatory bowel disease (IBD). Tsat, Transferrin saturation; sTfR, soluble transferrin receptor; CRP, C-reactive protein; RDW, red cell distribution width; RSF, Red blood cell size factor.

of them seem very promising in the evaluation of the anemia in patients with IBD, while others may have a role in reflecting anemia mechanisms. In any case, large studies are needed in order to validate them in everyday clinical practice.

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